AR201-14136

Randy_Deskin@GM.cytec.com on 12/18/2002 10:26:20 AM

To: oppt.ncic@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA
cc: Patti_Vernon@GM.cytec.com, Joseph_Caporossi@gm.cytec.com, Karen_Koster@GM.cytec.com

Subject: HPV Submission- CAS # 3748-13-8

Attached, find pdf format submission for m-Diisopropylbenzene CAS # 3748-13-8. Our submission includes a test plan and robust summary.

This completes Cytec's voluntary committment for the US HPV program for 2002.

A hard copy and diskette is also being submitted today.

Randy Deskin, Ph.D, DABT
Director, Toxicology and Product Regulatory Compliance

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CYTEC

CYTEC INDUSTRIES INC.

Toxicology & Product Regulatory
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December 18, 2002

Christine Todd Whitman
Administrator
U.S. Environmental Protection Agency
PO Box 1473
Merrifield, VA 22116
ATTN: Chemical Right-To-Know Program

Dear Administrator Whitman:

In keeping with Cytec's 1999 commitment to the EPA High Production Volume Voluntary program, attached find a robust summary and test plan for CAS # 3748-13-8, m-Diisopropylbenzene.

The robust summary and test plan is provided as hard copy and in .pdf format.

Sincerely,

Randy Deskin, Ph.D., DABT
Director, Toxicology and Product Regulatory Compliance Department

AR201-14136A

TEST PLAN FOR m-DIISOPROPENYLBENZENE (CAS NO. 3748-13-8)

OVERVIEW

Cytec Industries Inc. agrees to sponsor m-diisopropenylbenzene (CAS No. 3748-13-8) under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. The company hereby submits a test plan for this substance. It is the intent of the sponsoring company to use existing data discussed in the test plan combined with a new study for chromosomal aberration to fulfill the Screening Information Set (SIDS) endpoints.

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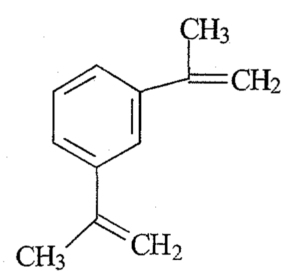
1. Introduction

Cytec Industries submits this test plan for hazard review under the Environmental Protection Agency High Production Volume Chemical Program. The technical contact at this company is:

Randy Deskin
Cytec Industries Inc.
Five Garret Mountain Plaza
West Paterson, NJ 07424
Phone (973) 357-3372

2. Designation of Test Substance

The test substance presented in this test plan is 1,3-diisopropenylbenzene (CAS No. 3748-13-8). Its chemical structure is as follows:



This substance is known by the following synonyms:

m-diisopropenylbenzene
meta-diisopropenylbenzene
benzene, 1,3-bis(1-methylethenyl)-
m-bis(1-methylvinyl)benzene
m-DIPEB (Cytec trade name)

The material will be referred to as m-diisopropenylbenzene in the test plan.

3. Criteria for Determining Adequacy of Data

All available studies were reviewed and assessed for adequacy according to the standards of Klimisch et al. (1997). Studies receiving a Klimisch rating of 1 or 2 were considered to be adequate. The m-diisopropenylbenzene test plan matrix (as shown in Table 1) was constructed after a careful evaluation of all existing data (see Sections 4.1- 4.46 below). This matrix is arranged by study type (columns) and screening data endpoints (rows), and indicates if data are provided for each end point in the set of robust summaries.

Table 1. Test Plan Matrix for m-diisopropenylbenzene

CAS No. 3748-13-8	Information	OECD Study	Other	Estimation	GLP	Acceptable	New Testing Required
ENDPOINT	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
PHYS/CHEM PROPERTIES							
Melting Point	Y	N	Y	N	N	Y	N
Boiling Point	Y	N	Y	N	N	Y	N
Density	Y	N	Y	N	N	Y	N
Vapor Pressure	Y	N	Y	Y[1]/N[2]	N	Y	N
Partition Coefficient	Y	N	Y	Y	N	Y	N
Water Solubility	Y	N	Y	N	N	Y	N
ENVIRONMENTAL FATE							
Photodegradation	Y	N	Y	Y	N	Y	N
Stability in Water	Y	N	Y	N	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	Y	Y	N	Y	N
Biodegradation	Y	Y	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	Y	N	Y	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	Y	N	Y	Y	N
Toxicity to Aquatic Plants	Y	Y	N	N	Y	Y	N
TOXICOLOGICAL DATA							
Acute Toxicity	Y	N	Y	N	Y[3]/N[4]	Y	N
Repeated Dose Toxicity	Y	N	Y	N	Y	Y	N
Genetic Toxicity-Mutation	Y	Y	N	N	Y	Y	N
Genetic Toxicity-Chromosomal Aberrations	N	N	N	N	N	N	Y
Toxicity to Reproduction	N	N	N	N	N	N	N[5]
Developmental Toxicity	N	N	N	N	N	N	N[5]
OTHER TOXICITY DATA							
Skin Irritation (NR)	Y	N	Y	N	N	Y	N
Eye Irritation (NR)	Y	N	Y	N	N	Y	N
Sensitization (NR)	Y	N	Y	N	Y	Y	N

Y = yes; N = no; NR = not required
[1] at 25 degrees C; [2] at > = 69.3 degrees C; [3] oral study; [4]inhalation and dermal studies; [5] not necessary because material is an industrial intermediate

4. Discussion of Available Test Information

4.1 Chemical and Physical Properties

The results of chemical/physical property testing are shown in Table 2.

Table 2. Chemical/physical properties of m-diisopropenylbenzene

Endpoint	Value
Melting point (° C)	-38 to -40 [a]
Boiling point (° C)	231 [a]
Vapor pressure (hPa)	0.1 (at 25° C) [b] 3.1 (at 69.3° C) [a] 990.6 (at 231° C) [a]
Partition coefficient (Log Pow or Kow)	4.89 [b]
Water solubility (mg/l at 25°C)	5.6 [a] 5.0 [b]

[a]measured; [b] estimated by EPIWIN

4.1.1 Melting Point

A measured melting point of -38 to -40°C was recently determined for m-diisopropenylbenzene (Rivera, C. 2002) following ASTM E-794, standard test method for melting and crystallization temperatures by thermal analysis. The purity of the test substance was 98.9%. The EPIWIN/MPBPWIN model estimates a value of -14°C.

4.1.2 Boiling Point

A measured boiling point of 231°C is listed on the Material Safety Data Sheet (Cytec Industries Inc., 2002). The purity of the m-diisopropenylbenzene was 100%.

4.1.2 Vapor Pressure

Vapor pressures have been measured for m-diisopropenylbenzene at several temperatures (Cytec Industries Inc., unpublished information). These values include a pressure of 3.1 hPa at 69.3° C and 990.6 hPa at 231° C. EPIWIN/MPBPWIN estimates a vapor pressure of approximately 0.1 hPa at 25° C, which is generally consistent with the measured value of 3.1 hPa at 69.3°C.

4.1.4 Octanol/Water Partition Coefficient

A log Kow value of ca. 4.89 was estimated by EPIWIN KOWWIN (v1.66), with the values for the CAS No. and boiling point (231 degrees C) being inputted. The program calculates the log Kow based on molecular structure and an algorithm that sums up individual contributions for the chemical fragments present in the molecule. This positive value is consistent with a non-polar aromatic substance with no water-soluble functional groups, which would be expected to have a high affinity for organic solvents, such as octanol.

4.1.5 Water Solubility

A measured water solubility value of 5.6 mg/l at room temperature has recently been determined for m-diisopropenylbenzene (Stanek, 2002). The purity of the test substance was 98.9%. The EPIWIN/WSKOW program estimated a water solubility of 5 mg/l based on an inputted log Kow of 4.89.

4.1.6 Summary/Test Plan for Physical Properties

Measured values are available for melting point, boiling point, vapor pressure and water solubility. The log Kow (partition coefficient value) was obtained using EPIWIN and is consistent with the molecular structure of the test substance and with its measured low water solubility value. The available data are sufficient to characterize the physical properties of m-diisopropenylbenzene as an organic liquid with relatively high boiling point, low vapor pressure and low water solubility. No further testing for these endpoints is planned.

4.2 Environmental Fate/Pathways

The results of environmental fate modeling and studies are summarized in Table 3 below.

4.2.1 Photodegradation

Photodegradation with hydroxyl radical sensitizer was estimated using EPIWIN/AOP (v1.90). An overall OH rate constant of ca 1.04×10^{-11} cm^3/(molecule*sec) was calculated based on the summation of individual rate constants for each bond fragment in the molecule using the program algorithm. A half-life of 1.225 hours was calculated assuming a constant concentration of OH radical and pseudo first order kinetics.

4.2.2 Stability in Water

An attempt was made to estimate the rate of hydrolysis for m-diisopropenylbenzene using the EPIWIN/HYDROWIN program. This estimation method, however, is valid only for molecules containing certain functional groups, including esters, carbamates, amides, and

Table 3. Environmental fate parameters for m-diisopropenylbenzene

Endpoint	Value
Indirect Photolysis (OH sensitizer) (Hydroxyl Radical Rate Constant)[a] (Atmospheric $T_{1/2}$)[a]	 ca 1.04×10^{-11} cm^3/(molecule*sec) 1.225 hours
Stability in Water	No reliable measured or estimated data[b]
Henry's Law Constant[a]	3.48×10^{-3} atm-m^3/mol
Koc[a]	4036

Environmental transport (Fugacity Level III mass percentages)[a]	Air = 0.214 Water = 24.9 Soil = 63.9 Sediment = 11.0
Biodegradation[c]	Not readily biodegraded

[a] Estimated using EPIWIN
[b]The test substance does not possess functional groups generally recognized to be readily hydrolyzable in water under neutral ambient conditions.
[c] Measured value

halomethanes. Measured hydrolysis data are not available. The test substance contains no functional groups generally recognized to readily undergo hydrolysis under neutral ambient conditions. Therefore, hydrolysis of this material is not likely to take place readily, especially at neutral ambient, conditions. Carbon-carbon double (olefinic) bonds are not readily attacked by water, but olefinic bonds can react with cold concentrated sulfuric acid via addition of a proton (H^+) followed by addition of sulfate anion (HSO_3^-) to form the alkyl sulfuric acid, which can then undergo hydrolysis to form the corresponding alcohol (Fieser and Fieser, 1957). However, since this reaction is not likely to occur in natural waters, one may conclude that hydrolysis of m-diisopropenylbenzene is unlikely to be an important degradative process in the environment.

4.2.2 Fugacity

Level III fugacity modeling has been conducted on the test material using the EPIWIN model. Measured inputs to the program are melting point (-39°C), boiling point (231°C), vapor pressure (1 mm Hg), and water solubility (5 mg/l). The value inputted for vapor pressure is the measured value extrapolated down to 25 degrees C. The results indicate that the test substance will partition in increasing preference to air, sediment, water and soil. A calculated Henry's Law Constant (the ratio of volatility to water solubility) of 3.48×10^{-3} atm-m^3/mol suggests that the test substance has some limited tendency to volatilize from water to the atmosphere. The value is consistent with the material having low volatility, but also having a limited affinity to water. A water soil partition constant (Koc) of 4036 has been estimated using EPIWIN PCKOC. This value indicates that the test substance possesses slight soil mobility.

4.3.4 Biodegradation

An OECD Guideline 301D (Close Bottle Test) has been conducted with 2 mg/l of a test material containing 97.5-99.1% m-diisopropenylbenzene (Drozdowski, 1987a). Under the conditions of the study, the test material did not biodegrade. At the concentration tested, the test material was not soluble. To increase surface area and immersion and reduce partitioning, the test material was applied to a carrier. Because the ability of the carrier to promote degradation of insoluble substances was not demonstrated with a reference material, the study was given a reliability rating of 2 (valid with restrictions). The results of this study are not inconsistent with what one might expect based on the molecular structure of the test substance. m-Diisopropenylbenzene is an aromatic substance with non-polar olefinic side chains. A substance with this structure has limited solubility in water, and possesses no functional groups that are readily vulnerable to biodegradation.

4.3.5 Summary/Test Plan for Environmental Fate Parameters

The environmental fate parameters discussed above indicate that the test substance, when released to the air, will readily undergo photodegradation (atmospheric half life ca 1.2 hours). However, some atmospheric material may be washed into the hydrosphere. Material released to water has some tendency to volatilize (Henry's Law Constant 3.48 x 10^{-3} atm-m^3/mol), but is expected to biodegrade slowly. As a result of limited solubility, material released to water in significant quantity is likely to be deposited in soil or sediment as well as air (due to some volatization). The material is not likely to be strongly persistent in the environment, because it does have some soil mobility (estimated Koc = 4036) and can volatilize slowly to the atmosphere, where it readily undergoes photodegradation. Sufficient data exist to characterize the environmental fate parameters at the screening level and no testing for these endpoints is planned.

4.3 Ecotoxicity

4.3.1 Acute Toxicity to Fish

A static GLP study in fathead minnows was performed with a material containing 99.13% m-DIPEB (Bowman, 1986). The no observable effect concentration (NOEC) and lethal concentration in 50% of the organisms (LC_{50}) in this 96-hour study were 1.2 and 6.2 mg/l, respectively. None of the fish exposed to $\leq$ 2.5 mg/l and all fish exposed to 20 mg/l died by 96 hours. This study was given a reliability rating of 2 (valid with restrictions) since concentrations of test material were not analytically confirmed and the results may have been influenced by insolubility of the test material at 10 and 20 mg/l.

4.3.2 Acute Toxicity to Aquatic Invertebrates

A static, GLP study in Daphnia magna was performed with a test material containing 99.13% m-diisopropenylbenzene (Forbis et al., 1986). The NOEC and LC_{50} values in this 48-hour study were 1 and 4 mg/l, respectively. None of the Daphnia exposed to $\leq$ 1.8 mg/l and all organisms exposed to 5.6 and 10 mg/l died by 48 and 24 hours, respectively. An oily film was present on the surface of water containing 5.6 and 10 mg/l, suggesting that the material might not be completely soluble at these concentrations. The study was given a reliability rating of 2 (valid with restrictions) since concentrations of test material were not analytically confirmed.

4.3.3 Acute Toxicity to Aquatic Plants

The toxicity of m-diisopropenylbenzene (98.3% pure) to Selenastrum capricornutum was tested according to OECD Guideline 201 (Drozdowski, 1987b). For this test, the index of toxicity is inhibition of growth rate. The NOEC, and effective concentration in 50% of the organisms (EC_{50}) at 96 hours were 1.77 and 4.92 mg/l, respectively. No growth occurred in cells exposed to 18 mg/l for 96 hours. In this study, there was no mention of the higher concentrations (10 and 18 mg/l) being insoluble. Based on results of the other aquatic toxicity tests, it is likely that the material was not completely soluble at these concentrations. Since concentrations of test material were not analytically confirmed, the study was given a reliability rating of 2 (valid with restrictions).

4.3.4. Summary/Test Plan for Ecotoxicity

Results of adequate studies in fathead minnows, Daphnia magna and Selenastrum capricornutum show that m-diisopropenylbenzene is of moderate toxicity to these species. For all the species tested, the average no effect concentrations and EC/LC_{50} values were approximately 1 and 5 mg/l. In all the studies, the LC_{50} values are likely to have been influenced by slight insolubility at the highest concentrations used (10 to 20 mg/l). As shown by Rivera (2002), the solubility limit of the material is 14 mg/l. Since the higher concentrations used in the aquatic toxicity studies did not appear to be completely soluble, the amount of test material available to the organisms at these concentrations was probably less than the nominal concentrations. Therefore, the actual LC_{50} values are likely to be slightly lower than those calculated. However, since concentrations of material listed as the no effect concentrations (NOECs) did not appear to be insoluble, and the material is not highly volatile, the nominal concentrations listed as the NOECs are likely to be similar to the actual concentrations present in the media. Since NOEC data appear to be valid, no additional testing is necessary.

4.4 Human Health Data

4.4.1 Acute Mammalian Toxicity

This endpoint is filled by two sufficient oral toxicity studies in rats (Calkins, 1981a; Chow, 1981a), two inhalation studies in rats (Myers, 1986; Nachreiner, 1986), and one dermal toxicity study in rabbits (Chow, 1981b). The oral and dermal LD_{50} values (lethal doses in 50% of the animals) were 13.2 ml/kg (approximately 12,200 mg/kg) and > 2000 mg/kg (the highest concentration tested), respectively. The LC_{50} value for aerosol inhalation was between 0.545 mg/l (the LC_0) and 5.576 mg/l (the LC_{100}). The purity of the test material used in all the acute studies was at least 97.5%. The oral study conducted by Calkins and the inhalation study conducted by Nachreiner are considered to be the critical studies for the endpoint, and were given reliability ratings of 1 (valid without restriction).

Clinical signs observed in rats treated orally with 5.0 to 20 ml/kg m-diisopropenylbenzene included diarrhea, lacrimation, lethargy, urine-soaked fur, nasal discharge, alopecia, crusty nose and eyes, and cold body temperature (Calkins, 1981a; Chow, 1981a). Four out of five males treated with 20 ml/kg exhibited alopecia/edema around the anus. Most of the signs were present only for the first days of the study (with the exception of alopecia, which generally appeared a week after treatment). The frequency or variety of signs did not appear to increase with increasing doses of test material, and did not exhibit any sex-related trends (with the exception of alopecia/edema around the anus of high dose males). The only effect noted in rabbits treated dermally with 2000 mg/kg m-diisopropenylbenzene after abrading the skin was slight dermal irritation (Chow, 1981b).

In rats exposed to 5.576 mg/l (5,576 mg/m^3) m-diisopropenylbenzene aerosol for 6 hours by inhalation, signs of toxicity such as wet fur, red perinasal wetness, lacrimation, whole body tremors, dermal irritation, hyperactivity, ataxia, and mouth breathing were observed during the first 90 minutes of exposure (Nachreiner, 1986). A complete loss of motor activity was observed in these animals for the remainder of the exposure period. After exposure, all animals exhibited absent toe, tail pinch, and surface righting reflexes, hypothermia, respiratory difficulties, wet fur, and dermal irritation. All of the animals appeared to be moribund before death (which occurred within 24 hours of exposure). In rats inhaling the nonlethal concentration (0.545 mg/l or 545 mg/m^3), ocular irritation occurred during exposure. By contrast, no signs of toxicity were observed in rats exposed to a saturated atmosphere of m-diisopropenylbenzene vapor for 6 hours (Myers, 1986).

4.4.2 Repeated Dose Mammalian Toxicity

A 28-day inhalation toxicity test (5 days per week for 4 weeks) with 107, 510, and 970 mg/m^3 m-diisopropenylbenzene (98.3% pure) has been performed in rats. Rats were predominantly exposed to vapor at 107 mg/m3 and approximately 50% vapor at 970 mg/m^3. The estimated percentage of respirable particles at 510 and 970 mg/m^3 was 86% and 89%, respectively. Exposure to 970 mg/m^3 m-diisopropenylbenzene was associated with decreased weight and weight gain in males, increased numbers of segmented neutrophils in the blood of both males in females, increased urine volume in males, increased liver weight, and increased concentrations of serum enzymes that are markers for liver toxicity. Effects observed at 510 mg/m^3 included reduced weight gain in males early on in the study, and increased urine volume and relative liver weight in males (without any changes in clinical chemistry parameters or pathology). Symptoms of eye irritation were observed in 1/10 animals exposed to 107 mg/m^3 and 6/10 animals exposed to 510 or 970 mg/m^3. Since study personnel did not consider the effects observed at 510 mg/m^3 to be indicative of systemic toxicity, they assigned a no observable adverse effect level (NOAEL) of 510 mg/m^3. However, since the effects observed at this concentration were also observed at 970 mg/m^3, they appear to be related to treatment. Since no systemic effects were observed at 107 mg/m^3, this concentration appears to be a more accurate estimation of the NOAEL.

4.4.3 Genetic Toxicity

4.4.3.1 Mutagenicity

m-Diisopropenylbenzene (98.36% pure) has been tested for mutagenicity in an OECD Guideline 471 study conducted with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and E. coli strain WP2uvra- (Thompson and Bowles, 1999). In these tests, the highest concentrations of m-diisopropenylbenzene that did not cause excessive toxicity were not mutagenic in the absence or presence of a metabolic activation system.

4.4.3.2 Chromosomal aberration

Since there are currently no data to fill this endpoint, testing is planned.

4.4.4 Reproductive and Developmental Toxicity

No reproductive or developmental toxicity tests with m-diisopropenylbenzene have been performed. m-Diisopropenylbenzene is used exclusively as an industrial intermediate, chemically converted to other products. The potential for significant human exposure is strictly limited. Therefore it is believed that this material qualifies for exemption from reproductive and developmental toxicity testing under the established guidelines of the HPV chemical program. Detailed documentation of the information required to substantiate manufacture and use as an industrial intermediate with limited exposure is provided in Appendix I of this test plan.

4.4.5 Additional Data

4.4.5.1 Skin and Eye Irritation

Adequate studies in rabbits show that 100% pure m-diisopropenylbenzene is slightly irritating to skin and moderately irritating to eyes (Chow, 1981b,c). Effects observed in the robustly conducted eye irritation study consisted of conjunctival redness, chemosis and/or discharge. Nasal discharge was observed in 3/9 animals a few days after exposure. No irritation to the cornea or iris was observed.

4.4.5.2 Sensitization

Results of a well-conducted GLP study in guinea pigs indicate that 100% pure m-diisopropenylbenzene is a sensitizer (Calkins, 1981b). Animals receiving induction applications of undiluted test material exhibited a dose-dependent dermal contact sensitization response when challenged with 100% test material and rechallenged with 12.5, 25, 50 and 100% test material.

4.4.6 Summary/Test plan for mammalian toxicity

Adequate studies with m-diisopropenylbenzene have been conducted for all endpoints except chromosomal aberration and reproductive/developmental toxicity. Acute oral, inhalation and dermal studies show that acute exposure to fairly large amounts of m-diisopropenylbenzene is required to cause lethality. Signs of nervous system toxicity are observed prior to death in rats exposed to 5,576 mg/m^3 m-diisopropenylbenzene by inhalation. Symptoms of toxicity observed in animals exposed to nonlethal concentratios of m-diisopropenylbenzene (by any route) are consistent with its ability to cause irritation to the skin and eyes. A well-conducted study in guinea pigs shows that m-diisopropenylbenzene is sensitizing. Results of the 28-day repeated dose inhalation study in rats show that exposure to 510 mg/m^3 m-diisopropenylbenzene produces adaptive changes in the liver (i.e. increased liver weight), and exposure to 970 mg/m^3 causes increased release of enzymes from the liver (but no pathologic changes). Adequate studies show that m-diisopropenylbenzene is not mutagenic or clastogenic.

Testing for chromosomal toxicity is advised since it has not been performed. Testing for reproductive/developmental toxicity is not necessary since m-diisopropenylbenzene is used exclusively as an industrial intermediate with low potential for human exposure.

5. Summary

In summary, valid data are present to satisfy all physical/chemistry, and environmental endpoints (with the exception of chromosomal aberrations and reproductive/ developmental toxicity). Testing for chromosomal aberrations is advised. Testing for reproductive/developmental toxicity is not necessary, since m-diisopropenylbenzene is used exclusively as an industrial intermediate that is chemically converted to other products, and has strictly limited potential for human exposure (See Appendix I for documentation). Existing studies on acute, repeated dose, and genetic toxicity (mutations) are sufficient to satisfy these endpoints. Data for eye and skin irritation and sensitization are adequate (although not required).

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APPENDIX 1: Documentation of Manufacture and Use of m-diisopropenylbenzene as an industrial intermediate

m-Diisopropenylbenzene is considered to be an industrial intermediate that meets the "type (c) description" in Chapter 2 of the OECD/SIDS Guidance Manual of "isolated intermediates with controlled transport, i.e. to a limited number of locations within the same company or second parties that use the chemical in a controlled way as an intermediate with a well-known technology." The following description follows Chapter 2 of the OECD/SIDS Guidance Manual for specific information required to support a claim for reduced testing for an isolated intermediate. This information has been provided by Cytec Industries Inc., the sole supplier for m-diisopropenylbenzene, and is accurate to the best of the company's knowledge.

m-Diisopropenylbenzene handled and used as an industrial intermediate, being chemically converted to other products. It is manufactured at one facility and used at two sites in the United States. A limited quantity is exported and used as an industrial intermediate at a third site.

Manufacture takes place using an enclosed, continuous process. The product is purified by distillation in closed, continuous equipment, and stored in tanks for a short time until needed for use. The manufacturing and distillation equipment is located outdoors, with operating controls indoors. The equipment is under vacuum, and is vented through a vacuum pump. Off-gases are combusted. This manufacturing system prevents any significant human exposure during manufacture.

All manufactured m-diisopropenylbenzene is transported in bulk (rail cars or tank trucks) from the manufacturing site to a Cytec facility. The rail cars are spotted over a dip pan to collect possible spillage during off-loading. The substance is transferred through closed lines into a closed storage tank. This tank is diked and dike area is checked on a regular basis to insure tank integrity. The tank is not open to the atmosphere, so no vapor release is expected.

Approximately 95% (roughly 2.5 million pounds) of this material is then transferred through closed lines into a reactor, where it is used as a raw material in the production of diisocyanate monomer. The reactor is located outdoors and is vented through a water scrubber system. The vented organics are water-soluble and are piped to the plant wastewater treatment. The primary opportunity for exposure is during sampling, but at that point the concentration of m-diisopropenylbenzene in the product stream is <1.0%. Workers are required to wear chemical resistant rubber gloves and safety glasses with side shields when sampling or carrying out other tasks. The product purification process removes unreacted m-diisopropenylbenzene, and analytical data on the final product normally shows no m-diisopropenylbenzene present at the limit of detection. Any material is collected in a process stream that is burned on site in an approved incinerator.

Less than 1% (ca. 20,000 pounds) of the m-diisopropenylbenzene is transferred from

Cytec in drums to a second facility in the U.S., and stored until required for use as intermediate for chemical conversion to other chemicals used in optical products. The m-diisopropenylbenzene is pumped from the drums into the reactor using closed lines. Drumming is performed in an open area outside, and workers are required to wear a hard hat, safety goggles and impervious gloves.

The remaining m-diisopropenylbenzene (<5% or ca., 100,000 pounds) is at the Cytec facility and is drummed for export as chemical intermediate in production of fragrances. The m-diisopropenylbenzene is pumped into a reactor using closed lines.

Although no industrial monitoring data are found for workplace concentrations of m-diisopropenylbenzene, such concentrations are believed to be low as a result of the closed equipment used during manufacture and conversion, the engineering controls described above and the substance's low vapor pressure (0.1 hPa @ 25 ° C). The number of workers potentially exposed is limited by manufacture at a single facility and conversion at three facilities; one of which (Cytec Industries) accounts for over 95% of its use. About 26 workers are associated with the manufacturing process, and approximately 21 workers for conversion at Cytec, Industries, Inc. A few additional personnel may be occasionally exposed to m-diisopropenylbenzene when sampling incoming rail cars, tank wagons and the storage tank.

AR201-14136 B

Robust Summaries and Dossier For m-Diisopropenylbenzene (CAS No. 3748-13-8)

Existing Chemical	:	ID: 3748-13-8
CAS No.	:	3748-13-8
Producer Related Part		
Company	:	Cytec Industries Inc.
Creation date	:	21.10.2002
Substance Related Part		
Company	:	Cytec Industries Inc.
Creation date	:	21.10.2002
Memo	:	
Printing date	:	17.12.2002
Revision date	:	17.12.2002
Date of last Update	:	17.12.2002
Number of Pages	:	39
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD AND COMPANY INFORMATION

Type	:	
Name	:	Cytec Industries Inc.
Partner	:	
Date	:	09.10.2002
Street	:	5 Garret Mountain Plaza
Town	:	07424 West Patterson, NJ
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Reliability	:	(1) valid without restriction

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

Substance type	:	organic
Physical status	:	liquid
Purity	:	> 98 % w/w
Reliability	:	(2) valid with restrictions

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

1,3-Diisopropenylbenzene
22.10.2002

Benzene, 1,3-bis(1-methylethenyl)-
22.10.2002
Benzene, m-diisopropenyl-
22.10.2002

m-Bis(1-methylvinyl)benzene
22.10.2002

m-Diisopropenylbenzene
22.10.2002

m-DIPEB
22.10.2002

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

Type	industrial
Category	Chemical industry: used in synthesis
Reliability	(2) valid with restrictions

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

No limits established

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	= -38 to -40 ° C
Decomposition	:	no at ° C
Sublimation	:	no
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4. Purity of test substance was 98.9%.
Method	:	ASTM E-794, Standard test method for melting and crystallization temperatures by thermal analysis.
Result	:	The E.O. (extrapolated onset) of enthalpy occurred at -42 degrees for the four runs. Peak enthalpy change took place at -38, -39, -39, and -40 degrees C for four runs.
Source	:	Cytec
Test condition	:	Duplicate samples were encapsulated in aluminum pans and heated in the Mettler 821 DSC. Each sample was heated from -150 degrees C to 10 degrees C at 10 degrees C/min. Nitrogen, flowing at -45 ml/min, purged the system during heating and cooling. The DSC was calibrated at 10 degrees C/min with an indium standard.
Reliability	:	(1) valid without restriction

03.11.2002 (22)

Value	:	ca. -14 ° C
Sublimation	:	
Method	:	other
Year	:	2002
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Inputs to the EPIWIN MPBPWIN program (v.1.40) were the CAS No. and a boiling point of 231 degrees C.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.

03.11.2002 (16)

2.2 BOILING POINT

Value	:	= 231 ° C at
Decomposition	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Methodological information was not provided on the MSDS. The purity of the test material was stated on the MSDS to be 100%.

(7)

2.3 DENSITY

Type	:	relative density
Value	:	= 0.925 at unknown temperature
Method	:	other
Year	:	
GLP	:	no data

Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions
Methodological information was not provided on the MSDS. The purity of the test material was stated on the MSDS to be 100%.

(7)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : ca. 0.1 hPa at 25° C
Decomposition :
Method other (calculated)
Year : 2002
GLP : No
Test substance : as prescribed by 1.1 - 1.4
Remark : Inputs to the EPIWIN MPBPWIN Program (v1.40) were the CAS No. and boiling point of 231 degrees C.
Reliability : (2) valid with restrictions
Data were obtained by modeling.

(16)

Value : = 3.1 hPa at 69.3° C
Decomposition :
Method other (measured)
Year : No data
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The following data are provided with respect to vapor pressures at various temperatures:

Degrees C	vapor pressure (torr)
69.3	2.3
86.7	5.3
101.6	11.5
111.9	17.5
123.4	26.5
134.2	41.0
144.7	58.7
151.8	78.6
161	107.3
166	129.3
172	148
231.2	743.1

Reliability : (4) not assignable
The method was not described and the purity was not given. No laboratory notebook reference or formal report or date of determination was given.

03.11.2002 (8)

2.5 PARTITION COEFFICIENT

Log pow : ca. 4.89 at ° C
Method other (calculated)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : Inputs to the EPIWIN KOWWIN Program (v1.66) were the CAS No. and a

boiling point of 231 degrees C.

Reliability : (2) valid with restrictions

(14)

2.6.1 WATER SOLUBILITY

Value : ca. 5.6 mg/l at ° C
Qualitative :
Pka : at 25 ° C
PH : at and ° C
Method : other
Year : 2002
GLP : No
Test substance : as prescribed by 1.1 - 1.4. Purity of the test substance was 98.9%.
Result : The highest m-DIPEB concentration dissolved in water was 5.6 ppm. Extractions of two samples showed m-DIPEB to attach to the glass container and float on the top of the water but not dissolve beyond 5.6 ppm.

Source : Cytec
Test condition : A minimum of five external standards of m-DIPEB were prepared in methylene chloride with ppm concentrations of 1.3 to 115. The percent relative standard deviation of the response factors (amount/area) for all calibrations was 2.1 to 2.9. Samples were transferred to a 2 liter separatory funnel by inserting a PFA tube to the bottom of the sample container. The samples were siphoned, discarding the first 150ml, into a 2 liter separatory funnel. Samples 2-2, 3-3, 7-2 were exceptions being poured into the separatory funnel to compare the upper portion of water and residue on the glass container. The samples were extracted with 40ml, 30ml, 30ml and 10ml of methylene chloride. The extraction was collected to the mark of a 100ml volumetric flask except sample 2-2, which was extracted with 200ml. The samples were analyzed by GC flame ion detector (FID) to determine m-DIPEB content.
Reliability : (1) valid without restriction

03.11.2002 (23)

Value : ca. 4.633 mg/l at 25 ° C
Qualitative :
Pka : at 25 ° C
PH : ca. 7 at and ° C
Method : other
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : Inputs to the EPIWIN WSKOW Program (v1.40) were the CAS No. and a boiling point of 231 degrees C.
Reliability : (2) valid with restrictions
Data were obtained by modeling.

03.11.2002 (18)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spect. : nm
Rel. intensity : based on Intensity of Sunlight
Indirect photolysis
Sensitizer :
Conc. of sens. :
Rate constant : ca. .0000000000104 cm^3/(molecule*sec)
Degradation : ca. 50 % after 1.225 hour(s)
Deg. Product :
Method : other (calculated)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : Inputs to the EPIWIN AOP Program (v1.90) were the CAS No. and a boiling point of 231 degrees C.
Reliability : (2) valid with restrictions
Data were obtained by modeling.

(12)

3.1.2 STABILITY IN WATER

Deg. Product :
Method : other (calculated)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : The test substance is an aromatic hydrocarbon with no functional groups readily subject to hydrolysis under neutral ambient conditions. It has low solubility and is expected based on its molecular structure to be resistant to hydrolysis.
Result : The EPIWIN HYDROWIN Program (v1.67) cannot estimate a hydrolysis rate constant for the test substance, because it does not contain functional groups recognized by EPIWIN for estimation.
Reliability : (4) not assignable

(13)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media :
Air (level I) : 0.214
Water (level I) : 24.9
Soil (level I) :
Biota (level II / III) : 11.0
Soil (level II / III) : 63.9
Method : Other

Year	:	2002
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN PCKOC estimates a Koc (water soil partition) constant of 4036. Inputs to the EPIWIN Level III fugacity model were the CAS No., a boiling point of 231 degrees C, a melting point of -39 degrees C, a water solubility of 5 mg/l and a vapor pressure of 1 mm Hg. The model inputted the following properties: Molecular weight = 158 g/mol Henry's Law constant = 0.00348 atm-m^3/mol (estimated) Log Kow = 4.89 (estimated) Temperature = 25 degrees C
Reliability	:	(2) valid with restrictions. Data obtained by modeling.

(15)(17)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other bacteria: activated sludge
Concentration	:	2mg/l related to Test substance related to
Contact time	:	28 day
Degradation	:	% after
Result	:	under test conditions no biodegradation observed
Control substance	:	aniline
Kinetic	:	% %
Deg. Product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	1987
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The test material was not soluble at the tested concentration. To compensate for this, the solution was micropipetted onto a disc of glass fiber filter, which was then added directly to the test vessel. This theoretically increased the surface area of the sample, limited surface film and escape resulting from water partitioning, and kept the sample immersed in the test bottle. Lower concentrations were not tested because the OECD guideline indicated that concentrations tested should be at least 2 mg/l. The test was valid, since the positive control biodegraded under the test conditions and the oxygen demand of the water and inoculum blanks did not exceed 5 to 10% of the anticipated theoretical value of the test material after 15 to 28 days.
Result	:	The TOD of the material (2 mg/l carbon) was 5.3 mg/l O_2. The average dissolved oxygen content of dilution water without inoculum (blank) on Days 0, 5, 15 and 28 was 8.5, 8.5, 8.3 and 8.1 mg O_2/l, respectively. The average dissolved oxygen content of dilution water with inoculum (inoculum blank) at these times was 9.0, 8.9, 8.0 and 8.1 mg O_2/l. The average dissolved oxygen content of dilution water with a carrier plus inoculum

(inoculum blank with carrier) on Days 0, 5, 15 and 28 was 9.0, 7.8, 7.4, and 7.5 mg O_2/l, indicating that the presence of the carrier increased oxygen consumption.

The average dissolved oxygen content of test vessels (those containing the carrier, test material and inoculum) on Days 0, 5, 15 and 28 was 9.0, 8.9, 7.6, and 7.4 mg O_2/l, which was not different from that of the inoculum blank with carrier. Therefore, the test material did not biodegrade.

The average dissolved oxygen content of the positive control (2 mg/l aniline with inoculum) on Days 0, 5, 15 and 28 was 9.0, 6.3, 3.5, and 2.2 mg O2/l, which was equivalent to 0, 42, 73 and 95% degradation.

Test condition : Dilution water was prepared by adding 1 ml each of the following solutions to 1 liter of distilled water: 1) 8.5 g/l KH_2PO4, 21.75 g/l K_2HPO_4, 33.3 g/l $Na_2HPO_4.2\ H_2O$, and 1.7 g/l NH_4Cl; 2) 22.5 g/l $MgSO_4.7H_2O$; 3) 27.5 g/l $CaCl_2$; and 0.25 g/l $FeCl_3.6H_2O$. The water was left at room temperature and gently agitated for 24 hours prior to use.

Test material was diluted with dilution water to provide a concentration of 2 mg/l. An aliquot of the test material was micropipetted onto a disc of glass fiber filter, which was then added directly to a test vessel partially filled with dilution water. The solution was then inoculated with 5 ml activated sludge from Bergen County, New Jersey, MUA (the numbers of bacteria were not stated), and the vessel was filled with dilution water. Dilution water was added by siphon to prevent air bubbles. After oxygen content was measured, the vessel was stoppered and sealed with a secondary cap and incubated in the dark at 20 +/- 1 degrees C for up to 28 days. Vessels containing 2 mg/l aniline (reference material) in dilution water and activated sludge (positive control), dilution water and inoculum with and without the carrier (inoculum blanks with and without the carrier) and dilution water with no inoculum (oxygen blank) were prepared similarly. Duplicate vessels were prepared for all test conditions (except the blank) for each time point that oxygen content was assessed (immediately, and after 5, 15 and 28 days). One vessel per time point was prepared for the blank.

At each time point (0, 5, 15 and 28 days), oxygen content of the medium was measured using a YSI dissolved oxygen analyzer 54A. Theoretical oxygen demand (TOD, NO_3) was calculated based on the empirical formula of the test material. The percent biodegradability was calculated as oxygen depletion (BOD mg/l)/[concentration of test material (mg/l) x TOD] x 100. The oxygen depletion of the sample was corrected by subtracting the value of the inoculum blank.

Test substance : The test material (CT-256-86) contained 97.5-99.1% m-DIPEB, 0.028 - 0.7% m-IPEC (m-Isopropenyl cumene, CAS No. 1129-29-9), 0 - 0.50% p-DIPEB (CAS No. 1605-18-1), 0 - 0.10% DIPB (1,3-Diisopropylbenzene, CAS No. 99-62-7), 0 - 0.07% m-IPES (m-Isopropenyl styrene; CAS No. 52780-24-2) and 0.2 - 1.8% unknowns.

Reliability : (2) valid with restrictions
Lower concentrations that were soluble should have been tested. It is assumed that the carrier would promote degradation by increasing surface area. However, this was not demonstrated. A positive control material that would be insoluble (and therefore would need a carrier) should have been used in the test instead of aniline (because aniline did not require use of the carrier).

29.10.2002 (11)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
NOEC	:	m = 1.2
LC50	:	m = 6.2
Method	:	other
Year	:	1986
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : None of the fish exposed to either of the controls or test material up to 2.5 mg/l died during the study. Ten and twenty percent of the fish exposed to 5 mg/l died by 72 and 96 hours, respectively. The mortality rate for fish exposed to 10 mg/l was 0% at 24 hours, 50% at 48 hours, 90% at 72 hours and 100% at 96 hours. The mortality rate for fish exposed to 20 mg/l was 0% at 24 hours, 40% at 48 hours, 70% at 72 hours and 100% at 96 hours. The 48 and 96 hour LC_{50} values (with confidence limits) calculated by the probit and binomial methods were 18 (12 - 69) mg/l and 6.2 (2.5 - 10) mg/l, respectively.

Six/ten fish exposed to 2.5 mg/l were quiescent at 96 hours. All fish exposed to 5 mg/l that survived exhibited abnormal behavior at 72 and 96 hours, which consisted of surfacing, quiescence, on bottom, or loss of equilibrium. Fish exposed to 10 or 20 mg/l exhibited these symptoms as early as 24 hours. Based on these data, the no effect concentration at 96 hours was 1.2 mg/l.

The temperature was 22 degrees C for all water samples. The dissolved oxygen concentration ranged from 9.1 - 9.2 at 0 hours to 5.8 - 7.3 at 96 hours. These values represented 105% to 66% saturation. There was no effect of test material on dissolved oxygen concentration. The pH ranged from 7.1 - 7.5. All temperatures, dissolved oxygen concentrations and pH values were within acceptable limits.

After stirring the solutions for 3 hours, the 5, 10 and 20 mg/l concentrations had a light film. The amount of film increased with increasing concentration. After 72 hours, the 10 mg/l solution still had a light surface film and the 20 mg/l solution had a heavier film.

Test condition : Test organisms: The fathead minnows used in the study were obtained from an in-house culture. All fish were on a 16 hour daylight photoperiod and observed for at least 14 days prior to testing. Fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii daily until they were transferred into the test vessels. The fish had a mean weight and length of 0.19 +/- 0.053 g and 24 +/- 1.6 mm, respectively. The loading biomass was 0.12 g/l for the definitive study.

Test material: Test concentrations of 0.60, 1.2, and 2.5 mg/l were obtained by transferring the appropriate volume of a working standard prepared in acetone to the test vessels. For test concentrations of 5, 10 and 20 mg/l, 1.5 ml of acetone was added to the appropriate weight of the dry material before addition to the vessels. The solvent control was a 1.5-ml aliquot of acetone.

Test water: The well water from which the reconstituted water was prepared contained < 20 ppb aluminum, <0.2 ppb arsenic, <2 ppb cadmium, <3 ppb chromium, <4 ppb cobalt, <3 ppb copper, 12 ppb iron, <

5 ppb lead, <0.5 ppb mercury, <15 ppb nickel, <5 ppb sliver, 11 ppb zinc, <0.10 ppb organophosphorus pesticides and <0.50 ppb organochlorine pesticides (including PCB's). The water was reconstituted to contain 48 mg/l $NaHCO_3$, 30 mg/l $CaSO_4.2H_2O$, 30 mg/l $MgSO_4$, and 2 mg/l KCl. The hardness, alkalinity and initial pH of the water were 40 - 45 mg/l (as $CaCO_3$), 30 - 35 mg/l (as $CaCO_3$) and 7.2-7.6, respectively. The dissolved oxygen concentration and pH at the start of the test were 9.1 mg/l and 7.4, respectively. The temperature of the water was kept at 22 +/- 1 degrees C.

Test conduct: Tests were conducted in 5 gallon glass vessels containing 15 liters of reconstituted water. The test fish (10 per test concentration) were acclimated to the dilution water for 48 - 96 hours prior to testing. They were not fed during this acclimation period or during the test. The test concentrations (0.6, 1.2, 2.5, 5, 10 and 20 mg/l) were chosen based on the results of a preliminary study performed with concentrations ranging from 1 to 320 mg/l. Two additional groups of 10 fish were exposed to dilution water alone or water containing the solvent. Each concentration was stirred for 3 hours before the fish were added randomly. All fish were observed at 24, 48, 72 and 96 hours for mortality and abnormal behavior. Dead organisms were removed after each observation. The pH, dissolved oxygen concentration and temperature of the control, solvent control and 0.60 ml test water were determined at the beginning of the test and after 48 and 96 hours. These variables were measured at 96 hours for water containing 5 mg/l and 0 and 48 hours for water containing 20 mg/l.

Statistical analysis: Concentration vs. lethality data were analyzed by a computer program which utilized the binomial, moving average and probit tests to determine the LC_{50} value (and 95% confidence limit). If no mortality occurred of if a dose-response could not be determined over a reasonable range (< 37 to > 63%), an LC_{50} value could not be calculated. The method of calculation selected for presentation was the one that gave the narrowest confidence limit.

Test substance : The purity of the test material (lot #S15183-124-1) was 99.13%. The composition of the remaining 0.87% is unknown.

Reliability : (2) valid with restrictions
The results at the two highest concentrations may have been influenced by insolubility of the test material. Test concentrations were not analytically confirmed.

29.10.2002 (1)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
NOEC : m = 1
LC50 : m = 4
Method : other
Year : 1986
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Result : None of the daphnids exposed to test material concentrations of 0, 1.0 or 1.8 mg/l died during the study. Two daphnids in one vessel containing 3.2 mg/l died between 24 and 48 hours. All ten fish exposed to this concentration in another vessel survived. Therefore, the overall death rate of daphnids exposed to 3.2 mg/l was 10%. Nine out of 10 daphnids exposed to 5.6 mg/l (both vessels) died within 24 hours, and all died by 48 hours. All daphnids exposed to 10 mg/l died within 24 hours. The 24 and

48 hour LC_{50} values (with confidence limits) calculated by the binomial method were 4.5 (3.2 - 5.6) mg/l and 4.0 (3.2 - 5.6) mg/l, respectively.

In one vessel containing daphnids exposed to 1.8 mg/l, 3 and 2 daphnids were surfacing at 24 and 48 hours, respectively. Approximately half of the daphnids exposed to 3.2 mg/l were surfacing and/or clumped at 24 hours. By 48 hours, the majority of daphnids exposed to this concentration had surfaced or were on the bottom of the vessels. Each of the surviving daphnids exposed to 5.6 mg/l for 24 hours were on the surface at this time period. The no observable effect level was therefore 1.0 mg/l at 48 hours.

The initial temperature, dissolved oxygen concentration and pH of the control water were 20 degrees C, 7.0 mg/l and 8.0. Whether this was the solvent or medium control was not specified. The temperature, dissolved oxygen concentration and pH of all water assayed at 48 hours were 20 degrees C, 8.7 - 9.1 mg/l and 8.4. All temperatures, dissolved oxygen concentrations and pH values were within acceptable limits.

An oily film was present on the surface of water containing 5.6 and 10 mg/l. This condition persisted throughout the experiment.

Test condition : Test organisms: The Daphnia magna used in the study were obtained from an in-house culture. The adults were fed algae (Selanastrum capricornutum) supplemented with a suspension of fish food at least every three days prior to testing. All daphnids were held at 20 +/- 2 degrees C, under a 16 hour daylight photoperiod (50-70 footcandles) with 30 minute simulated dawn and dusk periods. First instar daphnids (< 24 hours old) were used in the test. Test daphnids were not fed during the study.

Test material: Test concentrations were corrected for sample purity. A primary standard of 200 mg/ml was prepared by weighing 2.02 g and diluting it with 10 ml acetone. Appropriate volumes of this standard were added to test water (200 ml) to obtain test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg/l. Acetone (0.01 ml/200 ml test water) was the solvent control.

Test water: The water used in the study was from a deep well source. The water (1000 liters) was aged and activated biologically in a tank containing live fish. The water contained < 20 ppb aluminum, <0.2 ppb arsenic, <2 ppb cadmium, <3 ppb chromium, <4 ppb cobalt, <3 ppb copper, 12 ppb iron, < 5 ppb lead, <0.5 ppb mercury, <15 ppb nickel, <5 ppb silver, 11 ppb zinc, <0.10 ppb organophosphorus pesticides and <0.50 ppb organochlorine pesticides (including PCB's). The hardness, alkalinity, conductivity, dissolved concentration and initial pH of the well water were 225 - 275 ppm (as $CaCO_3$), 325 - 375 ppm (as $CaCO_3$), 700 micromhos/cm, 9.2 - 10.1 ppm, and 7.8 - 8.3, respectively. The temperature of the water was kept at 22 +/- 1 degrees C.

Test conduct: Tests were conducted in 250 ml glass beakers containing 200 ml of aged well water. The test organisms (10 per test concentration) were added randomly to the test water within 30 minutes of addition of test material. The test concentrations (1.0, 1.8, 3.2, 5.6 and 10 mg/l) were chosen based on the results of a preliminary study performed with concentrations ranging from 0.1 to 100 mg/l. Two additional groups of 10 organisms were exposed to dilution water alone or water containing the solvent. Each condition was tested in duplicate. All organisms were observed initially and after 24 and 48 hours of exposure for mortality and abnormal behavior (surfacing, clumping and lying on the bottom of the vessels). The pH, dissolved oxygen concentration and temperature of the control were determined at the beginning and end of the study. Water containing 1.0, 3.2 and 10 mg/l was analyzed for pH, dissolved oxygen concentration and temperature at the end (but not the beginning) of the study. The vessels were to be aerated if the dissolved oxygen level was less than or equal to 40% saturation.

Statistical analysis: Concentration vs. lethality data were analyzed by a computer program which utilized the binomial, moving average and probit tests to determine the LC_{50} value (and 95% confidence limit). If no mortality occurred of if a dose-response could not be determined over a reasonable range (< 37 to > 63%), an LC_{50} value could not be calculated. The method of calculation selected for presentation was the one that gave the narrowest confidence limit.

Test substance : The purity of the test material was 99.13%. The composition of the remaining 0.87% is unknown.

Reliability : (2) valid with restrictions
The results at the two highest concentrations may have been influenced by insolubility of the test material. Test concentrations were not analytically confirmed.

29.10.2002 (19)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
NOEC : m = 1.77
EC50 : m = 4.92
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1987
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Remark : Study personnel stated that "the use of the solvent produced a slight lag in the growth of cells but did not depress the population to a degree severe enough to confound the concentration effects".

Result : Cell counts given below are reported as the number of cells/ml x 10^4. Mean cell counts at 24 hours were reported as "less than 10" for all flasks. Average cell counts of controls at 48, 72 and 96 hours were 11, 75 and 241, respectively. Mean counts of cells exposed to 1.8 mg/l at 48, 72 and 96 hours were 14, 116 and 281, respectively. Inhibition of cell growth was noted with concentrations greater than or equal to 3.2 mg/l. The mean number of cells (and percent inhibition) of cells exposed to 3.2 mg/ml at 48, 72 and 96 hours were 11, 61 (19%) and 178 (26%), respectively. The data for one 3.2 mg/l flask were eliminated because the values at 48,72 and 96 hours (3, < 10 and 25) were considerably lower than the average. Numbers of cells exposed to 5.6 ppm (and percent inhibition) for 48, 72 and 96 hours were 10, 15 (80%) and 90 (63%). Inhibition of cell growth was observed as early as 48 hours for cells exposed to 10 and 18 mg/l (the results were "less than 10 x 10^4"). Cell counts (and percent inhibition) of cells exposed to 10 mg/l at 72 and 96 hours were 10 (87%) and 12 (95%). No growth occurred in cells exposed to 18 mg/l for 72 or 96 hours.

The rate of cell growth was satisfactory in the controls (greater than 16 x inoculum level at 72 hours) for acceptable data transformation. The correlation coefficients for the regression at 72 and 96 hours were 0.9 and 0.96, respectively. The no effect concentrations at 72 and 96 hours were 1.88 and 1.77 mg/l, respectively. The EC_{50} values at these times were 4.93 and 4.92 mg/l, respectively.

Test condition : Organisms: Selenastrum capricornutum (ATCC 2262) were propagated at 21 - 25 +/- 2 degrees C under 4000 lux illumination (continuous light). Stocks were subcultured on a regular basis (generally at 1-4 week intervals).

Three-five day old suspensions that yielded 1 x 10^4 cells/ml were used for the test. During the tests, algae were shaken, illuminated at 8000 lux, and maintained at a temperature of 22 - 22.5 degrees C.

Medium: OECD fresh water algal culture medium was prepared with distilled or deionized water in non-metallic containers and reconstituted with nutrients, salts and trace elements (as specified in the guideline). Medium was sterilized before use by filtration (<= 0.45 microns) or autoclaving.

Test material: The test material was diluted with anhydrous acetone to a concentration of 10,000 times the highest concentration to be used in the test. The stock was stored in the dark until used. Working standards in acetone were prepared so that 10 microliters of each standard would produce the desired concentration to be tested.

Test conduct: Based on results of a preliminary range-finding test, concentrations of 1.8, 3.2, 5.6, 10 and 18 mg/l were tested. Controls containing 10 microliters of acetone and 100 ml of algal suspension also were established. Each concentration (including control) was tested in triplicate. The initial and final pH of all media were recorded. The flasks were incubated for 96 hours and cells were counted with a hemocytometer daily.

A separate test was performed with untreated cells (medium control). The results were compared with those of the solvent control to determine if the solvent alone had an effect on cell growth.

Statistical analysis: The EC_{50} values at 24, 48, 72 and 96 hours were calculated by regression analysis, using the percent inhibition of growth calculated for each concentration. The percent inhibition of growth at each time was calculated by subtracting the mean cell count of test vessels (Tt) from that of controls (Ct), dividing the result by the Ct, and multiplying the result times 100. The data were graphed, and the no observed effect concentration was determined by extrapolation of the regression line or by data or graph inspection.

Test substance : The test material (CT-256-86) contained 97.5-99.1% m-DIPEB, 0.028 - 0.7% m-IPEC (m-Isopropenyl cumene, CAS No. 1129-29-9), 0 - 0.50% p-DIPEB (CAS No. 1605-18-1), 0 - 0.10% DIPB (1,3-Diisopropylbenzene, CAS No. 99-62-7), 0 - 0.07% m-IPES (m-Isopropenyl styrene; CAS No. 52780-24-2) and 0.2 - 1.8% unknowns.

Reliability : (2) valid with restrictions
Test concentrations were not analytically confirmed. Based on the results of other aquatic toxicity tests, it is likely that all test material at the highest two concentrations was not in solution.

29.10.2002 (10)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	100
Vehicle	:	no data
Value	:	= 13.2 ml/kg bw
Method	:	other
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The method of Litchfield and Wilcoxon could not be used to determine the LD_{50} value for males. It was estimated to be > 20 ml/kg because only one death occurred at this dose. Because the test for parallelism of dose-response curves or calculation of relative potency could not be carried out, the LD_{50} values for males and females combined could not be determined. Using a density of 0.925 (as stated in the MSDS), the LD50 value can be converted to 12.2 g/kg.
Result	:	Range finding study: None of the animals treated with < = 8.0 ml/kg died over the 7-day test period. One out of 2 females exposed to 10.0 ml/kg died the second day after treatment. Other animals given 10.0 ml/kg survived to termination. Many of the animals exhibited weight loss during the study. Weight loss did not exhibit any dose or sex-related trends. Clinical signs observed 24 to 72 hours after treatment included diarrhea, soft stool, wet area around anus, urine-soaked fur, lacrimation, nasal discharge, red nasal discharge, lethargy, crusty nose and face, swollen feet, paw cut, and moribund condition (for the rat that died). The symptoms increased in frequency with increasing concentrations of test material. There were no sex-related trends. Abnormal necropsy findings in treated animals included colon and/or cecum distended with gas (1 male and 1 female treated with 1.3 or 6.3 ml/kg, respectively), urinary bladder distended with reddish fluid (1 female treated with 10 ml/kg), yellow-green material in the stomach (1 female treated with 10 ml/kg), and yellow fluid in the ileum and cecum (1 female treated with 10 ml/kg). All control animals had normal necropsies. Main study: None of the animals treated with 0 (control) or 8 ml/kg died. The mortality rates of animals treated with 10.0, 12.6, 15.8, or 20.0 ml/kg were 1/10 (female), 3/10 (all females), 3/10 (all females), and 3/10 (1 male and 2 females). All animals that died succumbed between days 2 and 5. The LD_{50} value (and 95% confidence limits) was 13.2 (9.9-17.7) ml/kg for females and greater than 20.0 ml/kg for males. All animals (including controls) lost weight for a few days after dosing. Control and treated animals began to gain weight 48 and 96 hours after dosing, respectively. Total body weight gains over the 15 day period were similar between groups. Clinical signs in rats orally treated with 8.0 to 20 ml/kg m-DIPEB included diarrhea, lacrimation, lethargy, urine-soaked fur, nasal discharge, alopecia, crusty nose and eyes, and cold body temperature. Four out of five males treated with 20 ml/kg exhibited alopecia/edema around the anus. Most of the signs were present only for the first days of the study (with the exception of alopecia, which generally appeared a week after treatment). The frequency or variety of signs did not appear to increase with increasing doses of test material, and did not exhibit any sex-related trends (with the exception of alopecia/edema around the anus of high dose males). One male in the control group exhibited a crusty nose on day 8. All other animals assigned to the control group appeared normal.

In general, significant gross findings at necropsy were limited to animals found dead (mostly females). These included stomach (brownish-black material, distended with air, filled with yellow-green material, bright yellow fluid), intestinal tract (distended with dark brown material, filled with yellow-red material, yellow material, reddish fluid, yellowish-brown fluid and yellowish-brown material), urinary bladder (dark-colored fluid), and the carcass (alopecia, urine-soaked fur, autolysis, red material around the nose area and crusty eyes and face), and bright yellow nasal discharge and yellow material around nose. Findings in the lung (2 - 3 mm depressed area of one male treated with 15.8 ml/kg) and testes (red peduculated area in the fat of the epididymis of one male treated with 15.8 ml/kg) were considered incidental in nature.

Test condition : One hundred (50/sex) young adult TAC:N(SD)fBR rats were used for the study. Animals were quarantined and acclimated to laboratory conditions for a least a week prior to study initiation. They were individually housed in stainless steel cages with wire mesh floors. The cages were placed in a 6 cubic meter stainless steel and glass inhalation chamber. The chamber was well-ventilated (approximately 20 changes per hour), continuously monitored for temperature and humidity and had a controlled, 12 hr light/dark cycle. Food and tap water were available ad libitum (with the exception that rats were fasted overnight before dosing). All animals used in the studies were in good health.

A preliminary range finding test in which 2 animals/sex were intubated with 1.3, 1.6, 2.0, 2.5, 3.2, 4.0, 5.0, 6.3, 8.0 and 10.0 ml/kg was performed prior to the main study. Males and females used for range-finding weighed 251-329 g, and 201 - 246 g, respectively. For the main study, five animals/sex were intubated with 0.0 (control) 8.0, 10.0, 12.6, 15.8, and 20.0 ml/kg test material. Males and females used for the main study weighed 227 - 267 g, and 170 - 241 g, respectively.

Animals used in the range-finding study were observed for at least 4 days after dosing. They were weighed prior to treatment and 1, 2, 3, 4 and 7 days after treatment (at termination). Survivors were euthanized 7 days after treatment.

Main study animals were frequently observed for mortality and signs of toxicity during the day of dosing and twice daily thereafter. They were weighed prior to treatment and 1, 2, 3, 4, 7, 11 and 15 days after treatment (at study termination). All animals surviving to day 15 were euthanized.

In both studies, all animals that died and survived to study termination were subjected to a complete gross necropsy under the supervision of a board-certified veterinary pathologist as soon as possible after death.

The method of Litchfield and Wilcoxon was used to calculate the LD_{50} values (with confidence limits).

Test substance : The test material (#11583B14) was 100% m-DIPEB.

Reliability : (1) valid without restriction
The study conduct and documentation were robust.

Flag : Critical study for SIDS endpoint

29.10.2002 (3)

Type : LD_{50}
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle : no data
Value : > 5000 mg/kg bw
Method : other

Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : Two females were found dead on day 10. Labored breathing was observed in these rats just prior to death. Pathological findings included red hepatization and expended lungs, indicative of acute pneumonia or pneumonitis unrelated to treatment. There were no other deaths. Gross necropsies of survivors were unremarkable.

All animals exhibited soft feces, sedation, wet or crusty muzzle, and/or a wet peri-anal area after dosing. These symptoms also were observed 24 hour after treatment in most animals, but resolved within 6 days.
Test condition : Young adult Crl:COBS CD (SD) rats (5/sex) with acceptable body weights (weights were not stated) and general health were used. The rats were housed individually under a 12 hr light/dark cycle. Food and water were available ad libitum, except for an overnight fast prior to dosing. The animals were given a single oral dose of 5 g/kg test material (presumably by gavage). Animals were observed frequently during the day of dosing (Day 0) and twice daily for 14 days. All surviving animals were euthanized on Day 14 and examined grossly. Gross necropsies also were performed on animals that died during the study.
Test substance : The test material (CL 116,755) was 100% m-DIPEB.
Reliability : (2) valid with restrictions
Only one dose was tested. The condition of the animals was likely to have been influenced by the presence of a respiratory infection.
29.10.2002 (5)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC_{50}
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 20
Vehicle :
Exposure time : 6 hour(s)
Method : other
Year : 1986
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : The mean actual exposure concentrations (+/- SD) were 0.545 +/- 0.062 and 5.576 +/- 0.417 mg/l for the nominal concentrations of 3 and 15 mg/l. The MMAD and GSD for the two concentrations were 1.9 - 2.0 micrometers and 1.7, respectively.

All animals exposed to 5.576 mg/l died within 1 day of exposure. Signs of toxicity such as wet fur, red perinasal wetness, lacrimation, whole body tremors, dermal irritation, hyperactivity, ataxia, and mouth breathing were observed during the first 90 minutes of exposure to 5.576 mg/l. A complete loss of motor activity was observed in these animals for the remainder of the exposure period. After exposure, all animals exhibited absent toe, tail pinch, and surface righting reflexes, hypothermia, respiratory difficulties, wet fur, and dermal irritation. One high dose female also had an eye opacity. All high dose animals appeared to be moribund before death. Necropsies of the dead animals revealed discoloration of the lungs and kidneys and wet fur.

None of the animals exposed to 0.545 mg/l died. The only signs observed in rats exposed to this concentration were ocular irritation (blepharospasm and lacrimation) during exposure. Mean body weights for these animals were lower on Day 1 and higher on Day 5 than at the beginning of exposure. There were no gross lesions in these rats at necropsy.

The LC_{50} value was therefore > 0.54 and < 5.6 mg/l (or 540 or 5600 mg/m^3)

Test condition : Test article generation: The target nominal concentrations were 3 and 15 mg/l. An aerosol was generated with Laskin single-barrel nebulizer (for the 3 mg/l exposure) or a Laskin four-barrel nebulizer (for the 15 mg/l exposure). The nebulizer air pressure and air flow rate for the 3 mg/l exposure were 20 psig and 15 l/min and for the 15 mg/l exposure were 20 psig and 53 l/min. The chamber airflow for the 3 mg/l exposure was diluted with air to 60 l/min. The total volume of the test chambers was approximately 120 liters. The average temperature and relative humidity (+/- SD) of the low-concentration atmosphere were 20 +/- 0 degrees C and 30 +/- 2%, respectively. For the high-concentration atmosphere, these variables were 24 +/- 1 degrees C and 26 +/- 9%, respectively. Test atmosphere was sampled (using a filter) for 2 minutes at 35, 80, 125, 190, 245, 300 and 355 minutes into the exposure for the low concentration and 35, 95, 135, 185, 235, 285 and 340 minutes into the exposure for the high concentration. The concentration of test material was determined gravimetrically. The mass median aerodynamic diameter and geometric standard deviation (GSD) also were determined (method not stated).

Test conduct: Five rats/sex were exposed to each test atmosphere for 6 hours. Prior to exposure, the males and females weighed 188-239 and 150-171 g, respectively. The animals were observed during the exposure and 5 day recovery period (intervals were not stated). Weights were recorded on the day of exposure, one day after exposure and at termination (day 5). All animals were necropsied at death or at scheduled termination.

Test substance : The test material (CT-256-86) contained 97.5-99.1% m-DIPEB, 0.028-0.7% m-IPEC (m-Isopropenyl cumene, CAS No. 1129-29-9), 0 - 0.50% p-DIPEB (CAS No. 1605-18-1), 0 - 0.10% DIPB (1,3-Diisopropylbenzene, CAS No. 99-62-7), 0 - 0.07% m-IPES (m-Isopropenyl styrene; CAS No. 52780-24-2) and 0.2 - 1.8% unknowns.

Reliability : (1) valid without restriction
The study conduct and documentation were robust.

Flag : Critical study for SIDS endpoint

29.10.2002 (21)

Type : other
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Exposure time : 6 hour(s)
Method : other
Year : 1986
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : None of the animals died. There were no signs of toxicity during exposure to or after a saturated vapor of test material for 6 hours. No remarkable gross lesions were evident at necropsy. Animals gained weight during the 14 day recovery period.

Test condition : Animals: Rats weighed 200-300 grams and were approximately 5-8 weeks of age at the beginning of the study. They were acclimated for at least 5 days before exposure. Rats received food and water ad libitum (except during the exposure period). Five healthy rats/sex were used in the test.

Vapor generation: Approximately 100 grams of test material were placed into a large glass tray. The tray was placed in a 120-liter plexiglass chamber which was then tightly sealed. The sample was allowed to evaporate overnight. A mixing fan was operated for 30-minute intervals to thoroughly distribute the vapors. The temperature was maintained at 23 degrees C.

Test conduct: After approximately 18 hours of equilibration of the test material with chamber air, rats were placed into a gasketted drawer-type cage. The cage was quickly inserted through a specially sealed opening in the front of the chamber to minimize vapor loss. A separate chamber was used/sex. Oxygen was added (as needed) to maintain a chamber oxygen content of approximately 20%.

The rats were exposed to the atmosphere for 6 hours. They were observed at least once every 30 minutes during exposure. After the exposure period, the rats were placed in a well-ventilated area, examined carefully and returned to their normal housing quarters. Rats were examined twice a day for 14 days for signs of toxicity. Weights were recorded on the day of exposure and 7 and 14 days after exposure. All survivors were euthanized after 14 days and subjected to gross necropsy.

Test substance : The test material (CT-256-86) contained 97.5-99.1% m-DIPEB, 0.028-0.7% m-IPEC (m-Isopropenyl cumene, CAS No. 1129-29-9), 0 - 0.50% p-DIPEB (CAS No. 1605-18-1), 0 - 0.10% DIPB (1,3-Diisopropylbenzene, CAS No. 99-62-7), 0 - 0.07% m-IPES (m-Isopropenyl styrene; CAS No. 52780-24-2) and 0.2 - 1.8% unknowns.

Reliability : (2) valid with restrictions
The concentration of test material in the vapor was not determined analytically. Therefore, whether or not the atmosphere was actually saturated with vapor during exposure is unknown.

29.10.2002 (20)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 10
Vehicle :
Value : > 2000 mg/kg bw
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The study was subjected to a quality assurance audit. However, there is no indication that the study was performed according to GLP. The same study is described in Section 5.2.1 below (irritation). The study was given a reliability rating of 1 for irritation, since the one concentration tested was adequate for the endpoint.
Result : None of the animals died during the study, gross necropsies were normal, and all animals gained weight. The only effect of treatment was slight dermal irritation. Erythema scores of 1 were observed in all males and females on day 1, all males and 2 females on day 2, 3 males and 2 females on day 3 and 4 males and 1 female on day 4. An erythema score of 2 was noted in one female on day 3 and 2 females on day 4. One female had an erythema score of 3 on day 4. All edema scores were 0. The mean irritation scores for both sexes for days 1-4 were 1.0, 0.7, 0.7 and 1.2, respectively. All scores on days 7 and 14 were 0.
Test condition : Young adult rabbits (5/sex/dose) were randomly selected from a larger pool of animals with acceptable body weights (2340-2698 g for males and 2078-2788 g for females) and general health. Rabbits were individually housed

under a 12 hour light/dark cycle. Food and water were available ad libitum. The dorsal surface of all rabbits was clipped the day prior to dosing. Just prior to dosing, the skin was abraded in a lattice formation with a hypodermic needle drawn across the surface of the skin. Care was taken to penetrate the stratum corneum, but not the dermis. Test material was administered with a syringe at a dose of 2 g/kg (based on the specific gravity of the test material and the weight of the animal). The test material was held in place with an occlusive wrap secured by a bandage and elastic tape. The dressings were removed after 24 hours and the excess material was wiped off.

The animals were observed for signs of toxicity at 20 minutes, 1, 2 and 4 hours after dosing on day 0, and twice daily from days 1-14. Physical examinations were performed prior to dosing and on day 14. Body weights were recorded on days 0, 1, 2, 3, 6, 10 and 14. Dermal irritation was scored according to the method of Draize on days 1, 2, 3, 4, 7 and 14. The degree of erythema and eschar formation and edema were each scored on a scale of 0-4. All survivors were euthanized and subjected to a complete gross necropsy on day 14. Samples from treated and untreated skin were taken from the back of each animal and retained.

Test substance : The test material (CL 116,755) was 100% m-DIPEB.
Reliability : (2) valid with restrictions
Only one dose was tested.

29.10.2002 (4)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : occlusive
Exposure time : 24 hour(s)
Number of animals : 10
PDII :
Result : slightly irritating
EC classification :
Method : other
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The study was subjected to a quality assurance audit. However, there is no indication that the study was performed according to GLP. The same study is described above in Section 5.1.3 (acute dermal toxicity).
Result : None of the animals died during the study, gross necropsies were normal, and all animals gained weight. The only effect of treatment was slight dermal irritation. Erythema scores of 1 were observed in all males and females on day 1, all males and 2 females on day 2, 3 males and 2 females on day 3 and 4 males and 1 female on day 4. An erythema score of 2 was noted in one female on day 3 and 2 females on day 4. One female had a erythema score of 3 on day 4. All edema scores were 0. The mean irritation scores for both sexes for days 1-4 were 1.0, 0.7, 0.7 and 1.2, respectively. All scores on days 7 and 14 were 0.
Test condition : Young adult rabbits (5/sex/dose) were randomly selected from a larger pool of animals with acceptable body weights (2340-2698 g for males and 2078-2788 g for females) and general health. Rabbits were individually housed under a 12 hour light/dark cycle. Food and water were available ad libitum. The dorsal surface of all rabbits was clipped the day prior to dosing. Just

prior to dosing, the skin was abraded in a lattice formation with a hypodermic needle drawn across the surface of the skin. Care was taken to penetrate the stratum corneum, but not the dermis. Test material was administered with a syringe at a dose of 2 g/kg (based on the specific gravity of the test material and the weight of the animal). The test material was held in place with an occlusive wrap secured by a bandage and elastic tape. The dressings were removed after 24 hours and the excess material was wiped off.

The animals were observed for signs of toxicity at 20 minutes, 1, 2 and 4 hours after dosing on day 0, and twice daily from days 1 - 14. Physical examinations were performed prior to dosing and on day 14. Body weights were recorded on days 0, 1, 2, 3, 6, 10 and 14. Dermal irritation was scored according to the method of Draize on days 1, 2, 3, 4, 7 and 14. The degree of erythema and eschar formation and edema were each scored on a scale of 0 - 4. All survivors were euthanized and subjected to a complete gross necropsy on day 14. Samples from treated and untreated skin were taken from the back of each animal and retained.

Test substance : The test material (CL 116,755) was 100% m-DIPEB.

Reliability : (1) valid without restriction
The documentation for and conduct of the irritation study were robust.

29.10.2002 (4)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : .1 ml
Exposure Time :
Comment :
Number of animals : 9
Result : moderately irritating
EC classification : irritating
Method : Draize Test
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The study was subjected to a QA audit.
Result : No irritation to the cornea or iris was observed at any time point. Discharge, chemosis and/or redness of the conjunctivae were observed in most animals. The individual scores for each of these conditions were not listed; only the total Draize scores. The total Draize scores for unwashed and washed eyes ranged from 0 - 8 and 0 - 6 from days 1 - 4, respectively (out of a maximum score of 110). One animal with an unwashed eye experienced no irritation (all scores were 0). On day 7 one rabbit with an unwashed eye and another with a washed eye had scores of 2. All scores on days 10 and 13 were 0. Average scores for unwashed eyes on days 1, 2, 3, 4 and 7 were 2.6, 2.6, 3.6, 4.0 and 0.3, respectively. The scores for washed eyes at these times were 0.7, 2.0, 2.0, 3.3, and 0.7, respectively.

All animals gained weight over the study and none of them died. Nasal discharge was observed in one animal with unwashed eyes on day 2 and two animals with unwashed eyes on day 3.

Test condition : Test material (100 microliters) was placed in the cupped lower lid of the right eye of each of 9 male New Zealand White rabbits with acceptable body weights (2164 - 2852 g). A test with florescein conducted the day before instillation revealed that the animals did not have existing corneal injury. Six of the animals received no further treatment. The right eyes of the other three rabbits were rinsed with water for 60 seconds, 30 seconds after treatment. The animals were observed twice daily for general condition, behavior and signs of toxicity. Body weights were recorded on days 0, 6 and 14. The eyes were examined for discharge, chemosis, inflammation, and opacity according to the Draize method on days 1, 2, 3, 4, 7, 10 and 13. Food and water were supplied ad libitum. All rabbits were euthanized without necropsy on day 14.

Test substance : The test material (CL 116,755) was 100% m-DIPEB.

Reliability : (2) valid with restrictions
The test documentation did not list the individual scores for discharge, chemosis and/or redness of the conjunctivae.

29.10.2002 (6)

5.3 SENSITIZATION

Type : other
Species : guinea pig
Number of animals : 29
Vehicle :
Result : sensitizing
Classification : sensitizing
Method : other
Year : 1981
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Result : Range-finding study: All animals in the range finding study and those assigned to serve as non-sensitized controls at rechallenge exhibited weight loss over the 3 - 4 day observation period. Weight loss for these animals was considered to be the result of experimental stress. Other test animals that were weighed after more days on the study gained weight.

During this study, 1 male and 1 female assigned to the test material group were found dead. The female was found dead the second day of the second induction and the male was found dead the day after the third induction. At rechallenge, 1 female assigned to the non-sensitized primary irritation control group was found dead on the day after the last skin evaluation for rechallenge, just prior to being weighed. Whether these deaths were considered to be related to test material was not stated. No gross lesions were found in the male. The only female that died after the second induction had mottled lungs and the small intestine was distended with air. The necropsy data for the other female were missing.

For the range-finding study, no irritation was seen for test concentrations of 50% or below. Only 1/4 animals exhibited irritation at 100% (grade 3 and 2 erythema at 24 and 48 hours, respectively). Therefore the test material was applied at 100% during the induction and challenge phases.
At rechallenge, 12.5, 25, 50 and 100% test material was used. The only non-irritating concentration of DNCB was 0.01%. All other concentrations produced irritation in at least 2 animals. However, the highest concentration (0.1%) did not cause grade 2 irritation. Therefore, 0.1% DNCB was used during the induction phase and 0.01% was used at challenge and rechallenge.

Main study: For the main study, skin condition after the first application of 100% test material appeared normal. Skin reactions [erythema (avg. grade 3.3 - 4.0), edema (grade 1 - 2), eschar formation (grade 4 erythema), bleeding at the test site, fissures and/or desquamation] first appeared after the second application, peaked after the third application, and remained steady for the remainder of the induction period. Similar findings were observed for DNCB (with the exception that the severity scores peaked after the 5th application).

The average erythema score 48 hours after challenge with the test material (but not 24 hours after challenge) was higher than that of the range-finding study (1.7 vs. 0.5). For DNCB, the erythema scores after 24 and 48 hours of induction (1.9 and 1.2, respectively) were greater than those for the range-finding study (0 at both time points). All edema scores for test material and DNCB were 0 (with the exception of a grade 0.3 edema after 24 hours challenge with DNCB).

Twenty-four hours after rechallenge, average erythema scores of animals rechallenged with 12.5, 25, 50 and 100% test material were 0.6, 0.6, 1.5 and 1.3, respectively. The corresponding values for primary irritation control animals treated with these concentrations and examined after 24 hours were 0.3, 0.5, 0.3 and 0.5, respectively. Edema scores for rechallenged animals (ranged from 0.0 to 0.2, with no effect of concentration) were similar to primary irritation controls (ranged from 0-0.3). Forty-eight hours after rechallenge, average erythema scores of animals rechallenged with 12.5, 25, 50 and 100% were 0.2, 0.5, 0.7 and 1.6, respectively. The corresponding values for primary irritation control animals treated with these concentrations and examined after 24 hours were 0.0, 0.3, 0.8 and 1.5, respectively.

Compared to scores from DNCB-treated animals in the range-finding test (all were 0), erythema and edema scores of animals rechallenged with DNCB were greater at 24 (2.0 and 0.7, respectively) and 48 hours (2.0 and 0.5, respectively).

Test condition : Thirty-seven (19 males, 18 females) Hartley albino guinea pigs were used. Animals were acclimated to laboratory conditions for at least 7 days prior to study initiation. All animals used appeared healthy. Animals were randomly assigned to 5 different treatment groups.

On the day prior to each phase of the study, application sites on the dorsal surface of each animal were closely clipped with electric clippers and then shaved with a safety razor. This procedure was repeated as required. Each material was applied (0.5 ml) on a 1 x 1 Webril patch. The patches were held in place with Blenderm tape. The patches and entire trunk were wrapped with an impervious binder consisting of plastic wrap, gauze bandage, adhesive tape and masking tape. The patches were removed after 24 hours. After treatment, animals were maintained in inhalation chambers. Skin condition was evaluated upon patch removal and 24 hours later (48 hours after treatment).

Four animals/sex (325 - 426 g) were used for the range-finding study. In this study, 2 animals/sex were exposed to 100% test material and 1, 3, 10, 25 and 50% test material in 1% olive oil, and 2 animals/sex were exposed to 0.01, 0.025, 0.05 and 0.1% 1,2 dinitrochlorobenzene (DNCB) in alcohol (positive control). The patches were applied to the prepared site, with the patches containing the highest concentrations applied to the left side of the animal (highest at upper left) and the lowest concentrations applied to the right side (lowest at lower right). The highest concentration producing a mean score for erythema of less than 2 in the range-finding study was used for induction and challenge doses in the main study. A non-irritating dose of the DNCB was used as the positive control for the challenge phase of the main study.

Initially, 13 males and 12 females (349 - 504 g) were used for the main study. Eight males and 7 females were exposed to 100% test material for induction and challenge and 100% material and 12.5, 25 and 50% test material in olive oil for rechallenge. Five animals per sex were exposed to 0.1% DNCB in alcohol for induction and 0.01% DNCB for challenge and rechallenge. For induction, the test materials were applied to the appropriate test site 3 times per week on alternating days until a total of 10 applications were made. Skin condition was evaluated 24 and 48 hours after each application. Challenge doses were applied to previously unused sites 14 days after the last induction dose was applied. Skin condition was evaluated 24 and 48 hours after challenge.

All animals induced and challenged with test material were rechallenged with test material 11 days after challenge (2 concentrations per side), with the lowest concentration at the upper left and the highest at the lower right. DNCB was applied to the positive controls at a previously unused site. An additional 2 animals/sex (631 - 889 g) were added as controls for non-sensitized primary skin irritation. These animals were exposed to 100% test material or 12.5, 25 and 50% test material in olive oil at rechallenge only. Skin condition of all animals was evaluated approximately 24 and 48 hours after rechallenge.

All animals were observed for mortality and signs of toxicity twice daily. Body weights were taken before treatment and at termination. Animals found dead were to be necropsied as soon as possible by a board-certified veterinary pathologist. At challenge (for the test material and DNCB) and at rechallenge (for DNCB), mean scores for skin condition were compared to the mean scores found in the range-finding study. If the scores at challenge (and rechallenge for DNCB) were higher than those in the range-finding study, the material caused dermal sensitization. At rechallenge, mean scores of test animals and non-sensitized controls were compared. If the mean scores were higher in animals that had undergone induction than in those that had not (non-sensitized controls), the material caused dermal sensitization.

Test substance : The test material (#11583B14) contained 100% m-DIPEB.

Conclusion : Animals receiving induction applications of 100% test material exhibited a dose-dependent dermal contact sensitization response when challenged with 100% test material and rechallenged with 12.5, 25, 50 and 100% test material. The responses for the positive control DNCB also were positive.

Reliability : (1) valid without restriction
The test conduct and documentation were robust.

29.10.2002 (2)

5.4 REPEATED DOSE TOXICITY

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 4 weeks
Frequency of treatment : 6 hours/day, 5 days/week
Post obs. period : none
Doses : 107, 510 and 970 mg/m^3
Control group : yes
NOAEL : = 510
LOAEL : = 970
Method : other
Year : 1988
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Remark : Exposure concentrations were selected based on the results of a previous, 5-day range-finding study in male rats (5/group; 7 to 9 weeks old) with 380, 730, 920 and 1400 mg/m^3. Ocular and nasal discharge were observed during and after exposure to concentrations > = 730 mg/m^3. Mild body weight loss was noted in all groups, with no relationship to concentration. There were no treatment-related alterations in organ weights and no exposure-related necropsy findings.

The results of the range-finding study also indicated that the vapor concentration in an aerosol atmosphere of test material ranged from 375 to 478 mg/m^3 for total (vapor plus aerosol) concentrations ranging from 380 to 1400 mg/m^3.

For the main study, the particle size distribution of the 107 mg/m^3 atmosphere was not determined (protocol deviation) since there was an insufficient amount of aerosol present in the chamber at this concentration (i.e. most was vapor).

Study personnel did not consider the effects observed at 510 mg/m^3 [reduced weight gain in males early on in the study, increased urine volume in males, increased relative liver weight in males in the absence of any changes in clinical chemistry parameters or pathology, and swollen periocular tissue in males and females during exposure (but not at termination)] to be indicative of toxicity. Therefore, they assigned a no observable adverse effect level NOAEL of 510 mg/m^3. The summary preparer believes that based on the aforementioned effects at 510 mg/m^3, a NOAEL of 107 mg/m^3 is more appropriate.

Result : The mean actual exposure concentrations (+/- SD) were 107 +/- 13, 510 +/- 29 and 970 +/- 54 mg/m^3 for the nominal concentrations of 100, 500 and 1000 mg/m^3. The MMADs (and range) for the 510 and 970 mg/m^3 concentrations were 3.7 (2.8-4.5) and 3.7 (2.8-4.3) microns, respectively. The GSDs (and range) for these concentrations were 2.6 (1.9 - 4.0) and 2.3(1.8 - 3.0), respectively. The estimated percentage (and range) of particles <=10 microns for the 510 and 970 mg/m3 concentrations were 86 (78 - 91) and 89 (86 - 92), respectively. The mean daily chamber temperature and relative humidity for all groups ranged from 20 - 21 degrees C and 48 - 49%, respectively.

None of the animals died. Symptoms of eye irritation including lacrimation (one male exposed to 107 mg/m^3), swollen periocular tissue (3 per sex exposed to 510 mg/m^3 and 2 males and 4 females exposed to 970 mg/m^3), and periocular encrustation (one control and 2 males exposed to 107 mg/m^3) were observed during exposure. Perinasal encrustation was observed during the study in one male exposed to 107 mg/m^3 and one female exposed to 970 mg/m^3. The days at which these symptoms were observed were not listed. At termination, the incidence of conjunctivitis in the 0, 107, 510 and 970 mg/m^3 groups was 2/10, 2/10, 2/10 and 4/10. Two rats exposed to 1000 mg/m^3 had severe conjunctivitis. Two high dose females also exhibited alopecia of the head during the study (time was not indicated). There were no other treatment-related clinical signs.

Average body weights and weight gains of males exposed to 510 mg/m^3 were significantly lower than controls at day 4 (weight) and from days 0 - 4 and 0 - 11 (weight gains). Average body weight and weight gain of males exposed to 970 mg/m^3 were significantly lower than controls on days 4, 11, 18 and 25 (weights) and from days 0 - 14, 0 - 11, 0 - 18 and 0 - 25 (weight gains). Weights and weigh gains of females were similar to controls.

A statistically significant increase in the numbers of segmented neutrophils was observed in males and females exposed to 970 mg/m^3. This shift was not accompanied by an increase in total leukocyte count. A significant increase in ALT was observed in males (41% greater than control) and females (67% greater than control) exposed to 970 mg/m^3 test material. With respect to control, alkaline phosphatase increased by 38% and 43%, respectively, in males and females exposed to 970 mg/m^3. Total urine volume of males and exposed to 510 and 970 mg/m^3 and females exposed to 970 mg/m^3 increased (but was only significantly different from control for males).

Increases in absolute (females only) and relative (to body weight) liver weights were observed in high dose animals (males and females). High dose males also had increased relative (but not absolute) brain, adrenal and testes weights. Males exposed to 510 mg/m^3 also had increased relative liver weight.

There were no treatment-related gross or histological lesions at necropsy. Histological lesions included lymphocytic myocardities of unknown etiology (1 control male), renal cortical necrosis (one control male), and alopecia with pustular dematitis of the cervical skin (one 970 mg/m^3 female). Minimal to mild lesions of various respiratory tract tissues (such as clusters of macrophages in alveolar spaces and minimal-mild laryngitis and tracheitis) were seen in several rats of various groups with no relationship to treatment. Four male rats (two high dose and one each from the other treatment groups) had slight lung hemorrhage, which was believed to be an artifact of the euthanasia technique.

Test condition : Animals: Thirty-two male rats and 31 females [HSD:(SD)BR], 35 days of age, were received approximately 14 weeks before initiation of the study. Three male rats were euthanized on the day of receipt for quality control. The liver, submandibular lymph nodes, lungs, trachea, kidneys, heart, salivary glands and nasal turbinates and spleen were fixed and examined microscopically. The larynx was inadvertently missed (protocol deviation). Blood samples were obtained from 5 males for serology evaluation. Fecal samples were collected from 5 males and examined for intestinal parasites. Results of these tests plus physical examinations revealed that the rats were in good general health. Only animals that had body weights within 2 standard deviations from the group mean for each sex were eligible for use. Twenty animals/sex were randomly allocated to 4 exposure groups (5/sex/exposure). Animals were weighed and clinically examined prior to exposure. Food and water were available ad libitum during non-exposure periods. Animals were individually housed in stainless-steel wire-mesh cages (14 x 13.5 x 18 cm) during exposures and 2 - 3 per cage (23.5 x 20 x 18 cm) when not exposed. The animals were on a 12- hour light/dark cycle throughout the study.

Test article generation: The target nominal concentrations of vapor plus aerosol were 0, 100, 500 and 1000 mg/m^3. Single aspirator tubes were used to generate the 100 and 500 g/m^3 atmosphere and a double aspirator tube was used to generate the 1000 mg/m^3 atmosphere. Compressed air, supplied to each nebulizer, created a negative pressure causing the test material to be aspirated into the tubes and dispersed as a fine liquid aerosol. The liquid aerosol was then introduced into the top of the exposure chamber where it was diluted to the target concentrations and dispersed throughout the chamber with filtered air. The total volume of the test chambers was approximately 900 liters. The operating air pressures of the nebulizers used to generate the 100, 500 and 1000 mg/m^3 target concentrations were approximately 3, 7, and 8 psig, respectively. Chamber temperatures and relative humidities were recorded at least 11 times per exposure with a minimum-maximum thermometer and an Airguide Humidity Indicator.

Two midget impingers in series (each containing 15 ml of toluene) were used to scrub the chamber atmosphere for test material. Four samples were collected from each chamber during each 6-hour exposure. The sampling time for the 500 and 1000 mg/m3 concentrations was 20 minutes, and for the 100 mg/m^3 concentration was 60 minutes. Aliquots (1 microliter) from each impinger sample were analyzed by a gas chromatograph fitted with a flame ionization detector. The daily nominal concentrations were calculated by dividing the total amount of material delivered to the chamber by the total chamber airflow.

Particle size distributions of the aerosols were measured with a cascade impactor nine times (at least twice a week) during exposure to 500 or 1000 mg/m^3. The amount of material that collected on cellulose filters on the stages of the impactor was determined gravimetrically. The data were analyzed by probit analysis to obtain the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Study conduct: All animals were acclimated to the chambers (with filtered air only) for 2 days before exposure to test material. Groups of 5 rats/sex (48 days old) were exposed 6 hours/day, 5 consecutive days/week for 3 weeks to 0 (air only), 100, 500 and 1000 mg/m3. During the fourth week, all animals were exposed for 4 days and euthanized on the 5th day. The position of cages in the chambers was rotated weekly in a predetermined pattern.

All animals were observed prior to, during, and following each exposure for signs of toxicity. They also were observed once/day when not exposed. Before the first exposure and at termination, the anterior chambers of the eyes of each animal were examined by a veterinarian. All animals were weighed before the first exposure, and on days 4, 11, 18 and 25.

Urine was collected for approximately 15 hours on the day of termination. Food and water were available ad libitum. The total volume, color and turbidity, specific gravity, pH, occult blood, and concentrations of glucose, ketones, protein, bilirubin and urobilinogen were determined according to standard methods. Although not stated, it is assumed that urine was collected before blood.

Blood was obtained from the orbital sinuses of anesthetized animals at termination. Food was withdrawn during blood collection. Blood was analyzed for total erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count and prothrombin time. Leukocyte differential smears were prepared from rats in all groups, but were evaluated only for the control and high concentration groups. Reticulocyte smears were prepared for all groups, but were not evaluated. Serum was analyzed for creatinine, sodium, potassium, chloride, alanine aminotransferase (ALT), total protein, albumin, total, direct and indirect bilirubin, aspartate aminotransferase (AST), globulin, creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase, alkaline phosphatase and gamma glutamyl transferase.

All surviving animals were euthanized after blood and urine collection. The brain, liver, lungs, heart, adrenals and testes were weighed. Gross necropsies were performed and selected tissues were fixed in 10% neutral buffered formalin. The spleen, adrenals, brain, esophagus, parathyroids, heart, larynx, lymph nodes, testes, thyroid, eyes, ovaries, pituitary, muscle (gastroncnemius), nerve (sciatic) and gross lesions were examined histologically in controls and animals exposed to 1000 mg/m^3 animals. The bone marrow, lungs, nasal turbinates, thymus, kidneys, liver, and trachea were examined histologically for all groups.

Statistical analyses: Data for continuous variables were first analyzed for homogeneity using Bartlett's test. If Bartlett's test indicated heterogeneous variances, data were compared using an analysis of variance (ANOVA) for unequal variances. Medians and quartile deviations were calculated for non-parametric data. These data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test (as modified by Mann-Whitney). Homogeneous data were analyzed using ANOVA, followed by tests. The level of significance for all comparisons was $p < 0.05$.

Test substance : The test material (CT-256-86) contained 97.5-99.1% m-DIPEB, 0.028 - 0.7% m-IPEC (m-Isopropenyl cumene, CAS No. 1129-29-9), 0 - 0.50% p-DIPEB (CAS No. 1605-18-1), 0 - 0.10% DIPB (1,3-Diisopropylbenzene,

		CAS No. 99-62-7), 0 - 0.07% m-IPES (m-Isopropenyl styrene; CAS No. 52780-24-2) and 0.2 - 1.8% unknowns.
Reliability	:	(1) valid without restriction The test conduct and documentation were robust.
29.10.2002		(9)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	S. typhimurium strains TA1535, TA1537, TA98 and TA100
Concentration	:	1.5, 5, 15, 50, 150, 500, 1500 and 5000 micrograms/plate
Cytotoxic conc.	:	> = 150 micrograms/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	OECD Guide-line 471 "Genetic Toxicology: Salmonella typhimurium Reverse Mutation Assay"
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This test was conducted in conjunction with the E coli WP2urvA- test (see below).
Result	:	There was no increase in the number of mutants in any strain exposed to test material (with or without metabolic activation) in either test.

For test 1, the average number of mutants in control strains TA100, TA1535, TA98 and TA1537 (without S-9) were 107, 18, 24 and 9, respectively. The average number of mutants in strains TA100, TA1535, TA98 and TA1537 incubated with test material (without S-9) ranged from 72 - 115, 0 - 23, 16 - 26 and 4 - 14, respectively. The average number of mutants in control strains TA100, TA1535, TA98 and TA1537 (with S-9) were 115, 12, 35 and 19, respectively. The average number of mutants in strains TA100, TA1535, TA98 and TA1537 incubated with test material (and S-9) ranged from 76 - 115, 6 - 17, 26 - 38 and 14 - 20, respectively.

For test 2, the average number of mutants in control strains TA100, TA1535, TA98 and TA1537 (without S-9) were 76, 20, 28 and 10, respectively. The average number of mutants in strains TA100, TA1535, TA98 and TA1537 incubated with test material (without S-9) ranged from 61 - 78, 16 - 22, 20 - 27 and 8 - 14, respectively. The average number of mutants in control strains TA100, TA1535, TA98 and TA1537 (with S-9) were 76, 13, 28 and 20, respectively. The average number of mutants in strains TA100, TA1535, TA98 and TA1537 incubated with test material (and S-9) ranged from 62 - 85, 10 - 15, 24 - 34 and 15 - 21, respectively.

All of the positive controls induced at least a 3-fold increase in the frequency of revertant colonies compared to controls, thus confirming the sensitivity of the bacterial strains. The spontaneous mutation rates of the controls were acceptable. The results of the characteristics tests for all the strains were satisfactory. The S-9 mix was sterile.

The results of the preliminary toxicity study indicated that the test material was toxic to TA100 at concentrations > = 500 micrograms/plate. In the main study, the test material caused a visible reduction in the growth of the bacterial lawn beginning at 150 micrograms/plate (strain TA1535 without activation). Concentrations > = 500 micrograms/plate reduced the bacterial lawn in strains TA100 and TA1537 (with and without activation). A concentration of 1500 micrograms/plate reduced the lawn in strain TA98 (without activation) and caused 100% cell death in strain TA1535 (without activation). At 5000 micrograms/ml, the bacterial lawn of strain TA98 was reduced (with S-9).

Test condition : Bacteria: The Salmonella strains were obtained from the University of California at Berkeley. All strains were stored at -196 degrees C until use. Prior to use, characterization checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, and the spontaneous reversion rate. Overnight cultures were prepared in nutrient broth and incubated at 37 degrees C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titers determined by viable count analysis on nutrient agar plates.

S9 preparation: S9 was prepared from the livers of male Sprague-Dawley rats (250 g) approximately 1 month before the experiments were conducted. The rats received 3 consecutive daily doses of phenobarbitone/beta naphthoflavone (80 - 100 mg/kg/day) prior to liver removal. Before use, each batch of S9 was assayed for its ability to metabolize the indirect mutagens 2-aminoanthracene and benzo(a)pyrene. The S-9 was stored at -196 degrees C until use. The S-9 mix (5.0 ml S-9, 1.0 ml 1.65 M KCl/0.4 M $MgCl_2$, 2.5 ml 0.1 M glucose-6-phosphate, 2.0 ml 0.1 M NADPH, 2.0 ml 0.1M NADH, 25.0 ml 0.2 M sodium phosphate buffer, and 12.5 ml sterile water) was prepared aseptically immediately before the experiments and stored on ice. A 0.5 ml aliquot of S-9 mix and 2 ml of molten, trace histidine or tryptophan-supplemented top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated in triplicate on the day of each experiment.

Study conduct: Approximately half-log dilutions of the test material in dried dimethyl sulfoxide (DMSO) were prepared on the day of each experiment. Concentrations were corrected for purity (98.4%). Based on the results of a preliminary study, concentrations of 1.5, 5, 15, 50, 150, 500, 1500 and 5000 micrograms/plate were tested in triplicate for each strain (TA1535, TA1537, TA98 and TA100), with the exception that 1.5, 5 and 15 micrograms/plate were not tested in TA98 with S-9 and 5000 micrograms/plate only was tested in TA98 with S-9. Aliquots (0.1 ml) of the bacterial cultures were dispensed into test tubes followed by 2.0 ml of molten, trace histidine or tryptophan-supplemented top agar, 0.1 ml of the test material, vehicle (DMSO), or positive control [3 or 5 micrograms/plate N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) for TA100 and TA1535 without S-9; 80 micrograms/plate 9-aminoacridine (9AA) for TA1537 without S-9; and 0.2 micrograms/plate 4-nitroquinoline-1-oxide (4NQO) for TA98 without S-9; 1-2 micrograms/plate 2-aminoanthracene (2AA) for TA100, TA1535 and TA1537 with S-9; and 5 micrograms/plate benzo(a)pyrene for TA98 with S-9] and either 0.5 ml of S-9mix (for experiments with metabolic activation) or phosphate buffer (for experiments without metabolic activation). The contents of each tube were mixed and equally distributed onto the surface of Vogel-Bonner minimal agar plates (one tube per plate). All plates were incubated at 37 degrees C for approximately 48 hours and the frequency of revertant colonies was assessed using a colony counter. The test was repeated using the same experimental conditions.

The assay was considered valid if all tester strains exhibited spontaneous reversion rates similar to historical controls, if the appropriate characteristics for each strain were confirmed, all tester strain cultures contained 1 - 9.9 x 10^9 bacteria/ml, each positive control induced at least a 2-fold increase in mutants, there was a minimum of 4 non-toxic concentrations, and there was no evidence of excessive contamination. The test was considered positive if there was a reproducible, dose-related and statistically (Dunnett's method of linear regression) significant increase in the number of revertants in at least one strain.

Test substance : The test material (CT-664-99) contained 98.36% m-DIPEB. Impurities were 0.41% p-DIPEB (CAS No. 1605-18-1) and 0.65% unidentified material.

Reliability	:	(1) valid without restriction The test conduct and documentation were robust.
29.10.2002		(24)

Type	:	Bacterial reverse mutation assay
System of testing	:	Escherichia coli strain WP2uvrA-
Concentration	:	50, 150, 500, 1500 and 5000 micrograms/plate
Cytotoxic conc.	:	> 5000 micrograms/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This test was conducted in conjunction with the previously described Ames test.

Result : There was no increase in the number of mutants in E coli exposed to test material (with or without metabolic activation) in either test.

For test 1, the average number of mutants (without S-9) was 27 for the control and ranged from 25-33 for treated cultures. The average number of mutants (with S-9) was 32 in the control and ranged from 31 - 37 for treated cultures.

For test 2, the average number of mutants (without S-9) was 22 in the control and ranged from 16-26 for treated cultures. The average number of mutants (with S-9) was 28 in the control and ranged from 16 - 24 for treated cultures.

The positive control induced at least a 10-fold increase in the frequency of revertant colonies compared to controls, thus confirming the sensitivity of the bacterial strain. The spontaneous mutation rates of the controls were acceptable. The results of the characteristics test were satisfactory. The S-9 mix was sterile.

The results of the preliminary toxicity study indicated that the test material was not toxic to E. coli WP2uvrA- at the highest concentration tested (5000 micrograms/plate).

Test condition : Bacteria: E coli strain WP2uvrA- was maintained at -196 degrees until use. Characterization checks were carried out to confirm the uvrB or uvrA mutation and the spontaneous reversion rate. Overnight cultures were prepared in nutrient broth and incubated at 37 degrees C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titers determined by viable count analysis on nutrient agar plates.

S9 preparation: S9 was prepared from the livers of male Sprague-Dawley rats (250 g) approximately 1 month before the experiments were conducted. The rats received 3 consecutive daily doses of phenobarbitone/beta naphthoflavone (80 - 100 mg/kg/day) prior to liver removal. Before use, each batch of S9 was assayed for its ability to metabolize the indirect mutagens 2-aminoanthracene and benzo(a)pyrene. The S-9 was stored at -196 degrees C until use. The S-9 mix (5.0 ml S-9, 1.0 ml 1.65 M KCl/0.4 M $MgCl^2$, 2.5 ml 0.1 M glucose-6-phosphate, 2.0 ml 0.1 M NADPH, 2.0 ml 0.1M NADH, 25.0 ml 0.2 M sodium phosphate buffer, and 12.5 ml sterile water) was prepared aseptically immediately before the experiments and stored on ice. A 0.5 ml aliquot of S-9 mix and 2-ml of molten, trace histidine or tryptophan-supplemented top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated in triplicate on the day of each experiment.

Study conduct: Approximately half-log dilutions of the test material in dried dimethyl sulfoxide (DMSO) were prepared on the day of each experiment. Concentrations were corrected for purity (98.4%). Based on the results of a preliminary study, concentrations of 50, 150, 500, 1500 and 5000 micrograms/plate were tested in triplicate. Aliquots (0.1 ml) of the bacterial culture were dispensed into test tubes followed by 2.0 ml of molten, trace histidine or tryptophan-supplemented top agar, 0.1 ml of the test material, vehicle (DMSO), or positive control [2 micrograms/plate N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) without S-9 and 10 micrograms/plate 2-aminoanthracene (2AA) with S-9] and either 0.5 ml of S-9 mix (for experiments with metabolic activation) or phosphate buffer (for experiments without metabolic activation). The contents of each tube were mixed and equally distributed onto the surface of Vogel-Bonner minimal agar plates (one tube per plate). All plates were incubated at 37 degrees C for approximately 48 hours and the frequency of revertant colonies was assessed using a colony counter. The test was repeated using the same experimental conditions.

The assay was considered valid if the tester strain exhibited spontaneous reversion rates similar to historical controls, if the appropriate characteristics were confirmed, all tester strain cultures contained 1- 9.9 x 10^9 bacteria/ml, each positive control induced at least a 2-fold increase in mutants, there was a minimum of 4 non-toxic concentrations, and there was no evidence of excessive contamination. The test was considered positive if there was a reproducible, dose-related and statistically (Dunnett's method of linear regression) significant increase in the number of revertants.

Test substance : The test material (CT-664-99) contained 98.36% m-DIPEB. Impurities were 0.41% p-DIPEB (CAS No. 1605-18-1) and 0.65% unidentified material.

Reliability : (1) valid without restriction
The test conduct and documentation were robust.

29.10.2002 (24)

Test substance : as prescribed by 1.1 - 1.4

Remark : There are no in vitro experimental data for the chromosomal aberration endpoint.

5.6 GENETIC TOXICITY 'IN VIVO'

Test substance : as prescribed by 1.1 - 1.4

Remark : There are no in vivo experimental data for the chromosomal aberration endpoint.

5.7 CARCINOGENITY

5.8 TOXICITY TO REP RODUCTION

Test substance : as prescribed by 1.1 - 1.4

Remark : There are no experimental data for this endpoint.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test substance : as prescribed by 1.1 - 1.4

Remark : There are no experimental data for this endpoint.

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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